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W81XWH-16-1-0755

TITLE:

Peritransplant Treg-Based Immunomodulation to Improve VCA Outcomes

PRINCIPAL INVESTIGATOR:

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RECIPIENT: Children's Hospital of Philadelphia
Philadelphia, PA 19104

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have undertaken studies of how Treg cells may be used to enhance outcomes in VCA recipients, using murine models of orthotopic limb transplantation.

Using this model, we have shown that expansion of Tregs can be used to significantly prolong orthotopic limb allograft survival. In addition, we have shown that Treg infusion can be used to promote orthotopic VCA survival.

These data are highly encouraging and are of translational significance.

These studies are those proposed for Specific Aim 1, and Specific Aim 2 will be tackled in the coming year.

15. SUBJECT TERMS
allograft rejection, tolerance, costimulation blockade, T-regulatory cells

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	20	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

   This project uses murine models of orthotopic limb transplantation (Tx) to assess whether Treg-based cell therapies (Aim 1), or use of pharmacologic HDAC inhibitors (HDACi) that enhance Treg numbers and/or suppressive functions (Aim 2), can promote vascularized composite allotransplantation (VCA) survival.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

   vascularized composite allotransplantation, T-regulatory cells, HDAC inhibitors, Foxp3

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

   What were the major goals of the project?
   List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

   We have undertaken the Tasks of Specific Aim 1 and achieved the listed milestones.

Specific Aim 1: Can Treg cell therapy causes long-term orthotopic limb allograft survival?	**Months**
Major Task 1: Characterize impact of WT vs. HDAC-/- Tregs on orthotopic VCA survival	
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What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1: Can Treg cell therapy causes long-term orthotopic limb allograft survival?

Major Task 1: Characterize impact of WT vs. HDAC-/- Tregs on orthotopic VCA survival

• Subtask 1: Seek IACUC & ACURO approvals for Treg-based therapy in limb allograft model
Milestones Achieved: Approvals by local IACUC and ACURO were achieved.

• Subtask 2: Undertake Treg expansion, including characterization of suppressive function, and assessment of TSDR demethylation. • Subtask 3: Perform orthotopic limb allografts in conjunction with TCR mAb and/or WT or HDAC-/- Treg cell administration
Milestones Achieved: Efficacy of polyclonal WT vs. HDAC-/- Tregs on VCA survival

We began by using the strategy of IL-2 complex (IL-2C) administration as a way to boost Foxp3+ Treg numbers. **Fig. 1** shows how IL-2C administration increased Treg numbers in C57BL/6 mice. (A) IL-2C significantly increased the percentage of Foxp3+ Treg cells in the

splenic CD4+ T fraction, and total Foxp3+ Treg cell numbers (x10^6 cells/spleen); data (mean ± SD) with 4 animals/group/time-point, **p<0.01, ***p<0.005. A representative flow plot is shown at right with percentage of Foxp3+CD4+ Treg cells indicated. (B) IL-2C administration on days 0, 1 and 2 led to a peak in the percentage of Foxp3+CD4+ Tregs on day 5, with a decline thereafter towards baseline; data (mean ± SD) with 4 animals/group/time-point, *p<0.01, **p<0.01. (C) IL-2C administration did not affect Treg suppressive function as assessed *in vitro* assays (mean ± SD, n=4/group) using cells analyzed at day 5. (D) Western blots of Foxp3 protein expression in Tregs from mice treated with IL-2C or PBS (representative of 3 experiments).

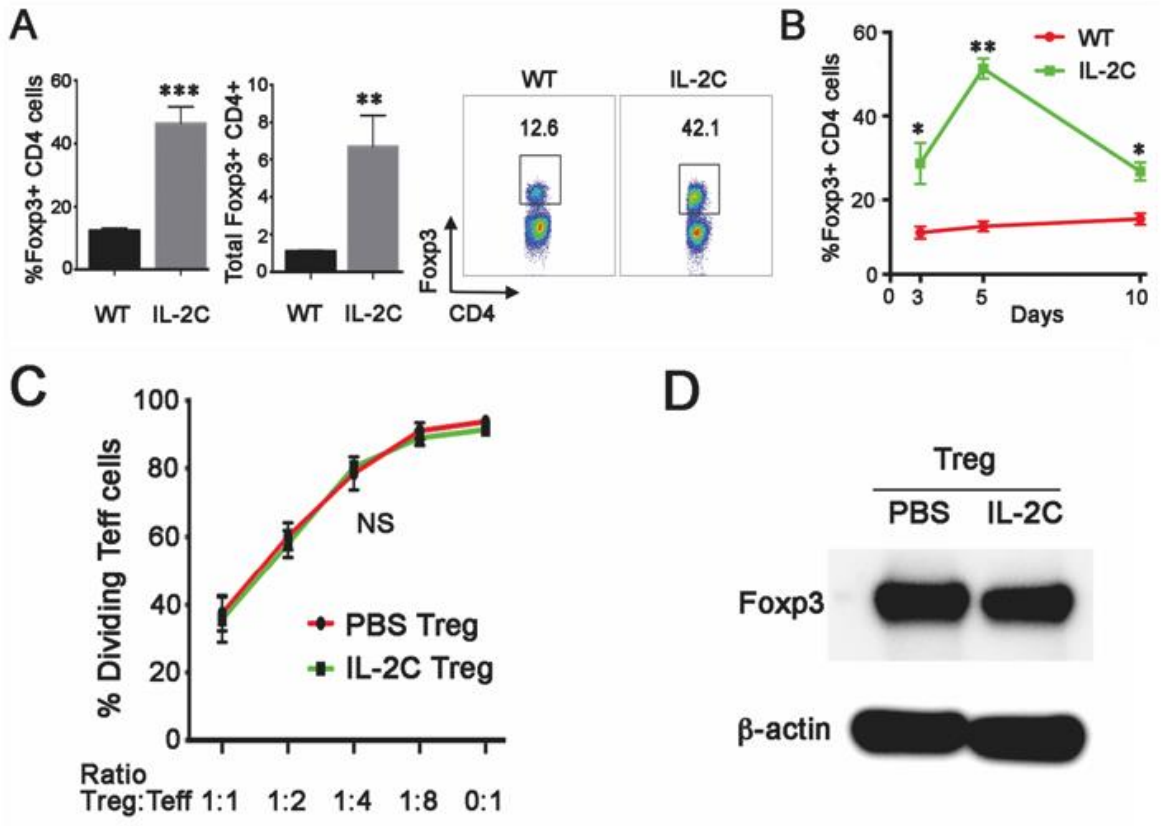


Fig. 1. Expansion and function of Foxp3+ Tregs.

We undertook assessment of Treg-specific demethylation region (TSDR) within the Foxp3 locus, by isolating Tregs and conventional T cells (Teff) from untreated and IL-2C treated B6 mice and undertaking bisulphite conversion, cloning and sequencing. WT Tregs were largely demethylated at the TSDR site (open circles, **Fig. 2**), whereas Teff cells were fully methylated (black circles) at the same site. Analysis of corresponding cells from IL-2C treated mice (day 5) showed comparable demethylation in Tregs but methylation in Teff cells. • ***Hence, IL-2C results in expansion of thymic-derived Tregs in the periphery of IL-2C treated mice, whereas on a per cell basis, Treg suppressive function is comparable to, but not greater than, that of untreated Foxp3+ Treg cells***.

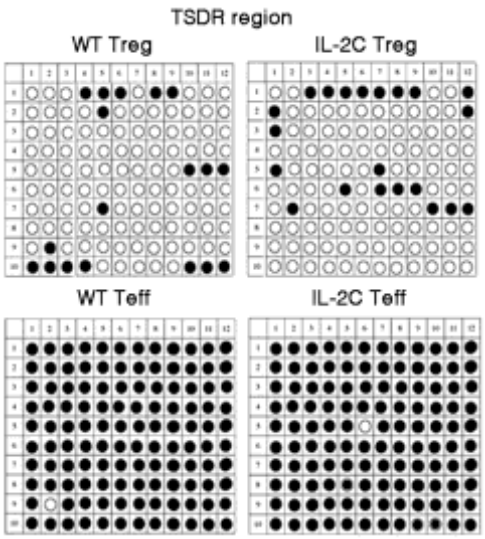


Fig. 2 Demethylation at the TSDR site.

We next tested effects of Treg expansion on VCA survival (**Fig. 3**). In initial studies (Fig. 3A) we tested the effects of combining post-Tx IL-2C therapy with administration of FK506 (1 mg/kg/d, i.p.) for 14 days from the time of transplantation. We found that post-Tx IL-2C therapy alone significantly prolonged VCA survival compared to the 3 other treatment groups ($p<0.01$); i.e. FK506 at this dose was ineffective in prolonging survival compared to untreated controls, and its combination with IL-2C therapy revoked the efficacy of the IL-2C regimen.

In subsequent studies, we tested the effects of IL-2C therapy alone or in conjunction with RPM therapy (2 mg/kg/d) delivered via 28 d Alzet pumps that were implanted beginning at the time of VCA engraftment. The experimental design is summarized in Fig. 3B, and comparisons between groups were undertaken at day 5 post-Tx. This point was selected given the onset of limb swelling and erythema by day 5 in untreated recipients. Rejection occurred by 10 days post-Tx in 50% of untreated recipients, and all allografts were rejected by day 12 post-Tx (Fig. 3C). Administration of IL-2C alone prolonged VCA survival, compared to untreated recipients, using both pre- and post-Tx protocols ($p<0.05$) (Fig. 3C), and administration of IL-2C post-Tx for longer periods, e.g. 5 days rather than 3 days had no additional benefit on VCA survival. Use of RPM monotherapy was about as effective as post-Tx IL-2C in prolonging survival ($p<0.05$, Fig. 3C).

Co-administration of IL-2C and post-Tx RPM had additional benefits, with pre-Tx IL-C plus RPM causing a 5-fold increase in survival, and post-Tx IL-2C plus RPM causing a 3-fold increase in survival, compared to untreated VCA recipients (Fig. 3). Comparison of intragraft events at day 5 post-Tx showed dense mononuclear cell infiltrates within the skin and muscle of grafts from untreated controls, along with areas of focal muscle necrosis (grade III rejection, Fig. 3D). Infiltrates were absent in recipients receiving pre-Tx IL-2C plus post-Tx RPM (grade 0, Fig. 3D), and were mainly confined to perivascular areas, without epidermal involvement or muscle necrosis, in recipients treated with post-Tx IL-2C plus RPM (grade I, Fig. 3D). The results of statistical comparisons of survival data for the various groups are shown in **Table 1**.

• ***We conclude from these data that while each therapy tested had benefit for graft survival, combinations of IL-2C plus RPM therapy were better, and pre-Tx IL-2C plus RPM resulted in the best overall prolongation of VCA survival and initial preservation of graft histology***.

Table 1. Kaplan-Meier analysis of allograft survival in the various experimental groups[1]

Group	Control	Post-Tx IL-2C	Post-Tx IL-2C+RPM
RPM alone	P<0.001	P=0.502	P<0.001
Post-Tx IL-2C	P=0.002	N/A	P<0.001
Post-Tx & IL-2C+RPM	P<0.001	P<0.001	N/A
Pre-Tx IL-2C	P<0.001	P=0.010	P=0.002
Pre-Tx IL-2C+RPM	P<0.001	P<0.001	P=0.010

[1] Comparison of survival curves (log-rank test, P value) using 6-8 allografts/group.

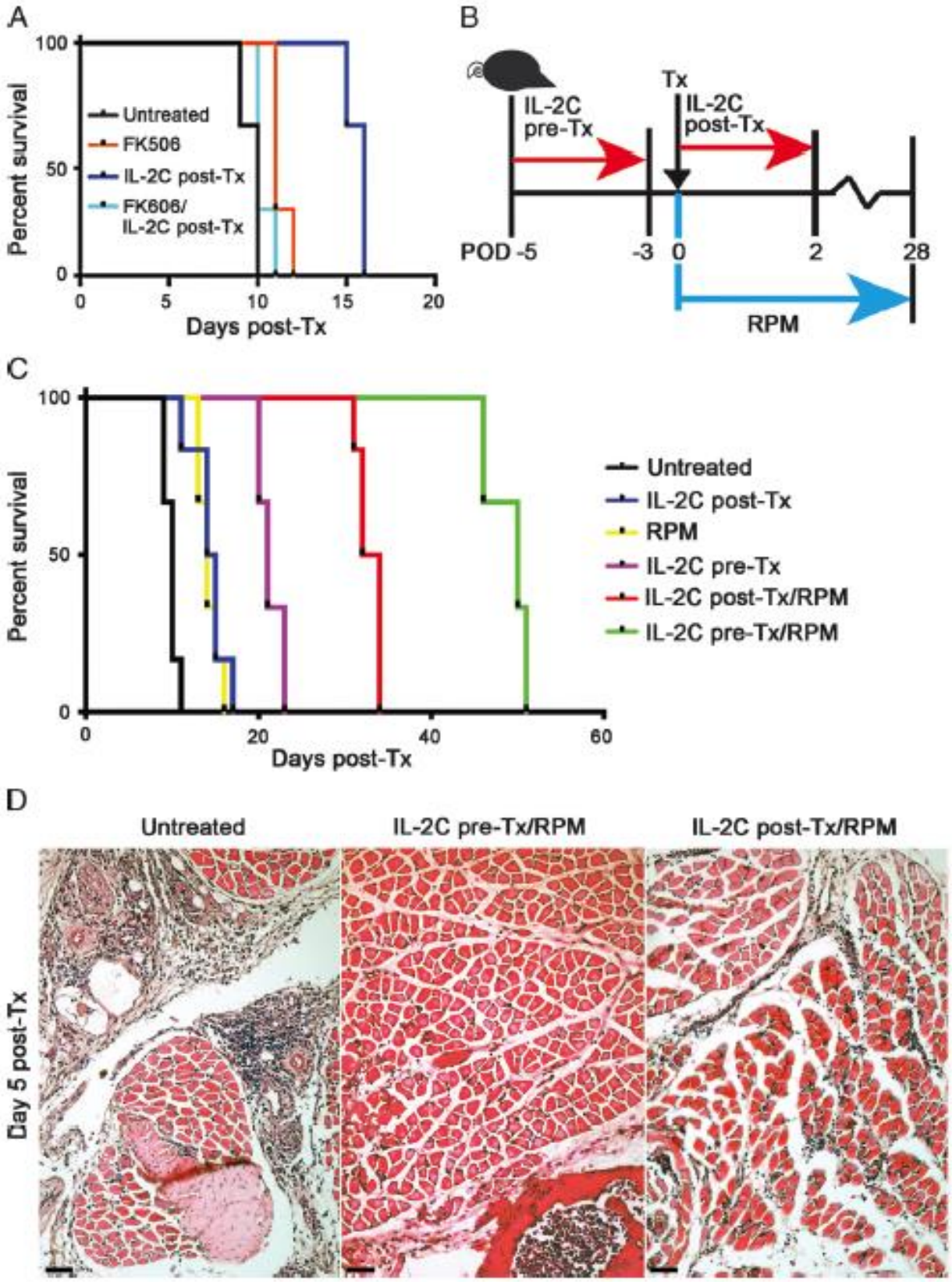


Fig. 3 Effects of Treg expansion on VCA survival.

We next assessed the effects of IL-2C therapy on host Treg and Teff cells (**Fig. 4**). At day 5 post-Tx, the proportions of Foxp3+ CD4+ Treg cells in recipients treated with IL-2C alone, or IL-2C plus RPM, were about 4-fold higher than in untreated allograft recipients ($p<0.05$), and about 2-fold higher than in mice treated with RPM alone (Fig. 4A). Mice treated pre-Tx with IL-2C ($p<0.05$) ± post-Tx RPM ($p>0.05$ vs. RPM alone) had lesser increases in Treg cells (Fig. 4A). However, at day 5 post-Tx, Tregs isolated from all 6 groups of engrafted mice showed comparable levels of IL-10 (Fi. 4B), GITR, ICOS and TGF-β, and comparable levels of cell proliferation (Ki67 expression) (Fig. 4C).

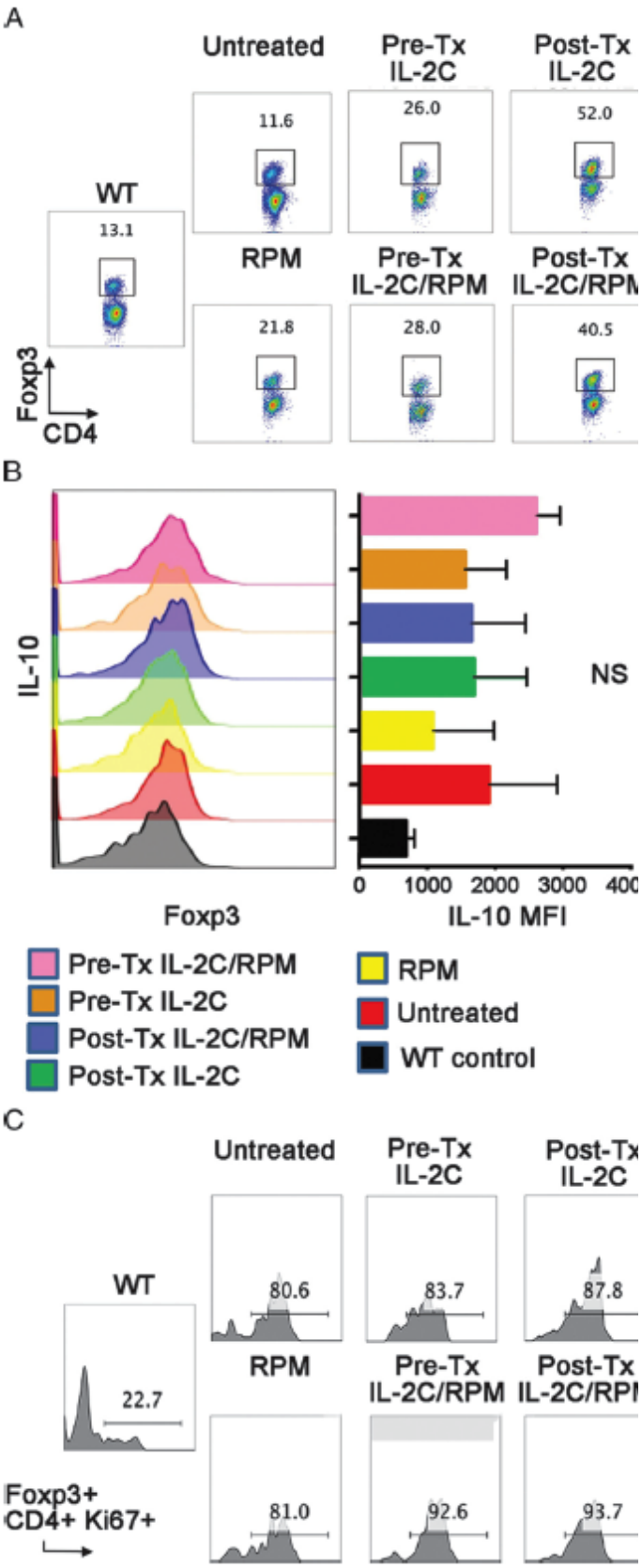


Fig. 4 Effects of IL-2C on Tregs vs. Teff cells (day 5 post-Tx)

Flow cytometric analysis of conventional CD4 and CD8 T cells, at day 5 post-Tx (**Fig. 5**), showed comparable proportions of CD4 cells in untreated recipients and those receiving pre-Tx IL-2C ± RPM (Fig. 5A). However, allograft recipients receiving post-Tx IL-2C ± RPM showed a 3-4 fold expansion of the CD8 population (Fig. 5A). Analysis of Ki67 expression showed increased proliferation of CD4 (Fig. 5B) and especially CD8 T cells (Fig. 5C) in all allograft groups compared with WT controls. This increase in proliferating CD8 T cells was most marked in recipients receiving post-Tx IL-2C, and in contrast to the other groups receiving RPM, was not diminished by post-Tx RPM therapy (Fig. 5C). Analysis of IFN-γ production by CD4 (Fig. 5D) and CD8 T cells (Fig. 5E) showed increases in all groups compared to WT controls, but was greatest in the case of recipients receiving post-Tx IL-2C and was diminished but not abolished by concomitant RPM therapy (Fig. 5E). Flow cytometric comparisons of the ratios of proliferating Tregs to CD4 or CD8 T cells at day 5 post-Tx (Fig. 5F) showed that the pre-Tx IL-2C/RPM protocol was especially effective at facilitating Treg expansion while curtailing CD4 and CD8 alloproliferation. In contrast, the groups receiving post-Tx IL-2C ± RPM showed particularly low Treg to CD8 T cell ratios.

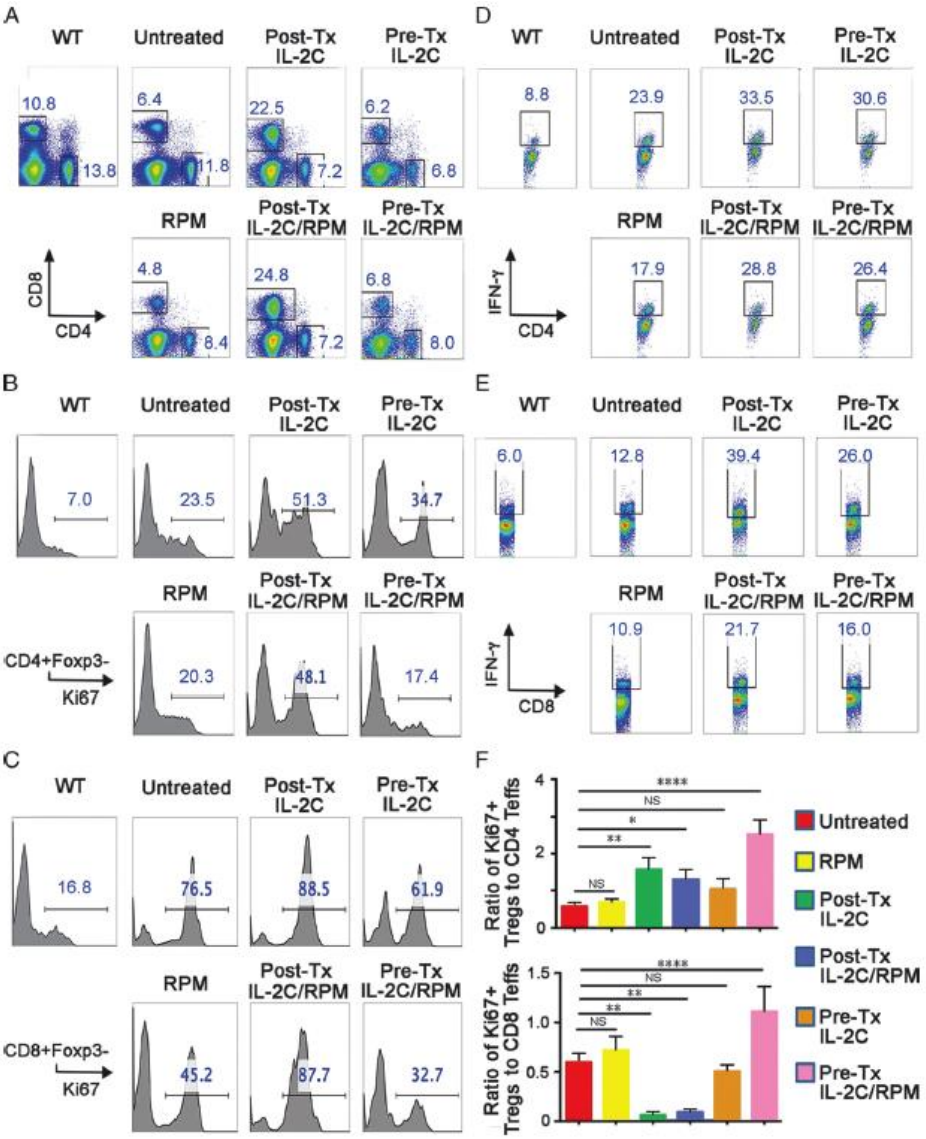


Fig. 5 Effects of IL-2 on non-Treg cells at day 5 post-Tx.

• ***These data indicate important differences in the levels of alloreactive CD8 T cells in VCA recipients receiving the post-Tx IL-2C, regardless of added RPM therapy, compared to pre-Tx IL-2C usage***.

Analysis of intragraft gene expression at day 5 post-Tx showed that, compared to pre-Tx IL-2C therapy, post-Tx IL-2C usage increased intragraft CD8, IFN-γ and granzyme B expression (**Fig. 6**). Addition of RPM decreased expression of CD8, IFN-γ and granzyme B in the post-Tx IL-C group, but was especially effective in decreasing expression of these genes in recipients treated with IL-2C in the pre-Tx period. Foxp3 and IL-10 gene expression were increased in all groups receiving IL-2C therapy, and levels were only modestly decreased by RPM therapy.

• ***These data suggest that at the level of the graft, as with events in secondary lymphoid tissues, post-Tx IL-2C therapy was less effective than pre-Tx therapy in controlling alloreactive CD8 T cell responses***.

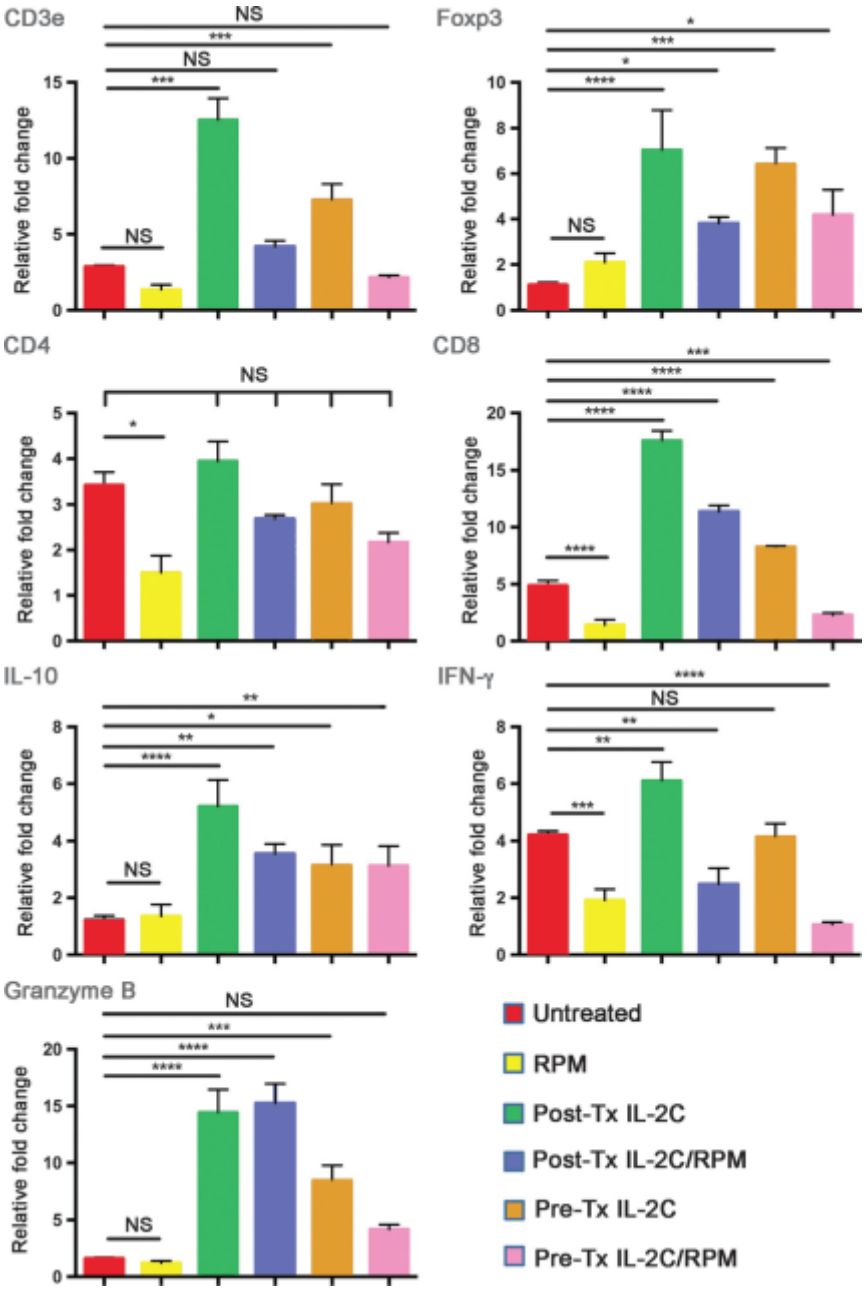


Fig. 6. Real qPCR analysis of intragraft gene expression (day 5 post-Tx, 4/group).

With regard to successfully achieving Major Task 1, the data shown in Figures 1-6 show that polyclonal Treg expansion can, indeed, be used to significantly prolong VCA survival.

Major Task 2: Effects of donor-specific WT vs. HDAC-/- Tregs on orthotopic VCA survival

• Subtask 1: Undertake donor-specific Treg expansion in vitro, prior to their infusion in vivo, including characterization of suppressive function, and assessment of TSDR demethylation for each population (WT Tregs, HDAC6-/- Tregs, HDAC11-/- Tregs).

Donor-specific Tregs were generated using FACS-purified YFP+ Foxp3+ B6 (H-2^b) Tregs that were cultured at $5x10^5$ cells/ml and stimulated with Dynal beads coated with anti-CD3/CD28 (4:1 bead: cell ratio) for 7-14 d plus IL-2 (500 IU/ml) and donor APC ($5x10^5$/ml) of BALB/c (H-2^d) or third party C3H (H-2^k) origin.

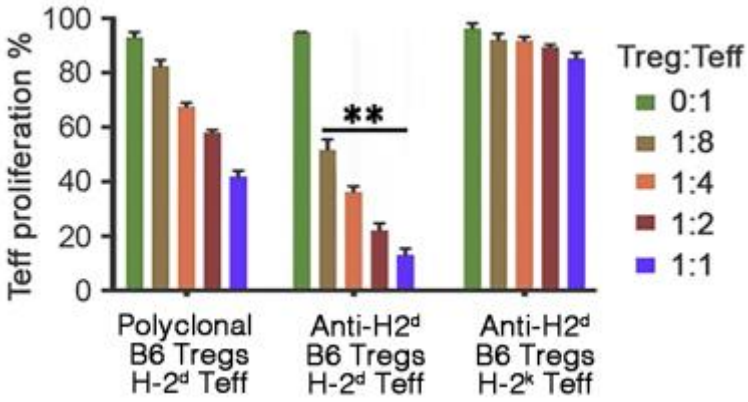


Fig. 7. Treg suppression assays using WT B6 Tregs (polyclonal) and BALB/c (H-2d) Teff cells (left); donor-specific B6 Tregs and donor Teff (H-2^d) cells (middle); or third party (H-2^k) T eff cells (right). Data are shown as area-under-curve (AUC) and 3 samples/group; **p<0.01 vs. either WT or third-party responses.

Treg suppressive function post-culture was used to compare suppression against donor (H-2^d) vs. third party (e.g. H-2^k) Teff cells. As seen in **Fig. 7**, using H-2^d Teff cells, donor-specific (anti-H-2^d) Tregs showed enhanced suppressive function, whereas these cells showed minimal suppressive function when tested against third party H-2^k Teff cells. Hence, these Tregs are significantly more suppressive than naïve C57BL/6 Treg cells (AUC analysis) against donor but not third party (C3H, H-2^k) cells.

• Subtask 2: Undertake VCA in conjunction with donor-specific WT or HDAC-/- Treg administration.

We undertook BALB/c-> B6 orthotopic VCA and infused recipients at the time of engraftment with polyclonal B6 Tregs, donor-specific Tregs, or Tregs specific for third party MHC. As seen in **Fig. 8**, donor-specific Tregs were significantly more effective (p<0.01) than WT or third-party Tregs at prolonging VCA survival.

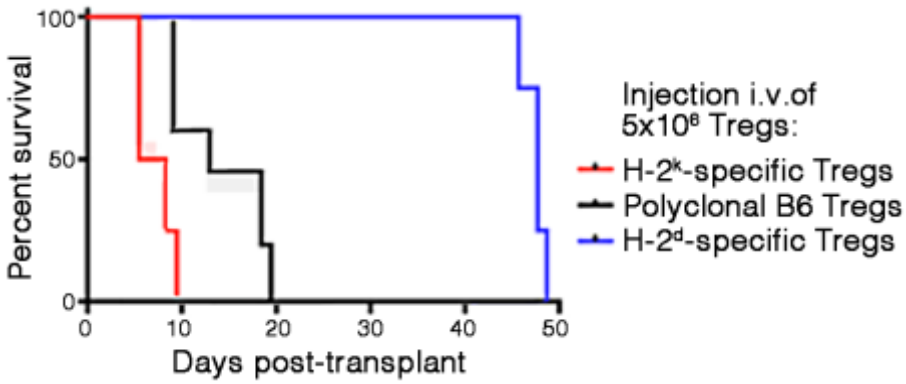


Fig. 8. Effects of infusion of polyclonal, donor-specific or third-party specific Treg infusion on VCA survival (BALB/c->C57BL/6, H-2^d->H-2^b).

Milestones Achieved: Efficacy of donor-specific vs. WT Tregs on VCA survival was shown.

With regard to successfully achieving Major Task 2, the data shown in Figures 7-8 show that donor-specific Tregs significantly prolong VCA survival.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Test the effects of HDAC targeting using HDAC inhibitors and/or Tregs from HDAC KO mice on VCA survival, alone or in conjunction with TCR mAb or RPM (i.e. Specific Aim 2).

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge,

theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Xu H, Dahiya S, Wang L, Akimova T, Han R, Zhang T, Zhang Y, Qin L, Levine MH, **Hancock WW**, Levin LS. Utility of IL-2 complexes in promoting the survival of murine orthotopic forelimb vascularized composite allografts.
  Transplantation (In press)

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report.

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  <u>Presentations</u> by Dr. Hancock

12/2016	"Novel Immunomodulatory Strategies for VCA" Department of Defense Fort Detrick, MD
7/2017	"An Update on Novel Immunomodulatory Therapies for VCA" Department of Surgery, Duke University Durham, NC

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Wayne Hancock, MD, PhD
No change

Liqing Wang, MD, PhD
No change

L. Scott Levin, MD
No change

Matthew Levine, MD, PhD
No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Attached (next page)

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Peritransplant Treg-Based Immunomodulation to Improve VCA Outcomes

DoD Idea Discovery Award W81XWH-16-1-0755

RT150100

PI: Wayne W. Hancock **Org:** Children's Hospital of Philadelphia **Award Amount:** $450,000.00

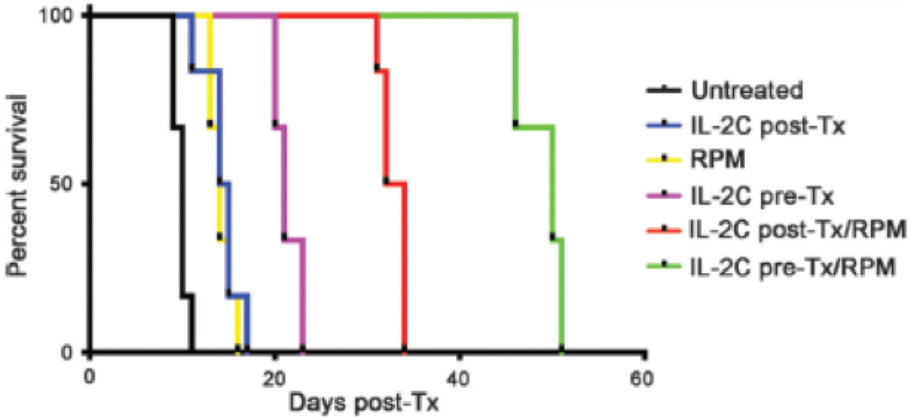
Study/Product Aim(s)

• **Aim 1** - Determine if Foxp3+ T-regulatory (Treg)-based cell therapy can promote long-term murine limb vascularized composite allotransplantation (VCA) survival.

• **Aim 2** - Determine if histone/protein deacetylase (HDAC) inhibitor -based pharmacologic modulation of Tregs will cause long-term VCA survival.

Approach

We propose proof-of-principle studies in murine VCA models with wild-type Treg cells or with Treg cells that have enhanced suppressive function as a result of specific deletion of one or more histone/protein deacetylase (HDAC) enzymes, followed by translational studies testing the effects of one or more courses of therapy with pharmacologic inhibitors of the corresponding HDACs in wild-type (WT) VCA recipients.



We have established a fully MHC-mismatched murine limb VCA model (BALB/c->C57BL/6). Treg expansion *in vivo* is achieved using 3 days of IL-2/anti-IL-2 mAb complexes (IL-2C). IL-2C & rapamycin (14 d therapy) pre-Tx was superior to the same therapy given post-Tx.

Timeline and Cost

Activities CY	16	17	18	
Regulatory approval & begin VCA				
Test effects of Treg cell therapy				
Test effects of HDACi therapy				
Publish results				
Estimated Budget ($K)	**$000**	**$225K**	**$225K**	

Updated: Oct 27, 2017

Goals/Milestones (Example)

CY16 Goal – Obtain regulatory approval and establish VCA model

☑ IACUC and ACURO approval

CY17 Goals – Test effects of Treg cell therapy on VCA survival

☑Investigate effects of WT & HDAC-/- Tregs on VCA survival

☑Investigate effects of donor-specific WT & HDAC-/- Tregs on VCA survival

CY18 Goal –Test effects of HDACi therapy on VCA survival

☐Test effects of TCR mAb treatment ± HDAC6i or HDAC11i therapy on VCA survival

☐Determine if the benefits of HDACi therapy are Treg dependent

☐Publish the results of our studies

Comments/Challenges/Issues/Concerns

- No concerns.
- Spending is on track

Budget Expenditure to Date

Projected Expenditure: $225,000 (direct)

Actual Expenditure: = $225,000 (direct)